Effects of Perinatal Exposure to Bisphenol A on Sociosexual Behavior of Female and Male Rats

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Perinatal action of estrogens or aromatizable steroids at the central nervous system level is responsible for brain sexual differentiation. Through early contact with the central nervous system, the estrogenic compound bisphenol A (BPA) could alter the processes affecting sociosexual behavior. To test this hypothesis, we studied agonistic and sexual behavior of adult female and male rats whose mothers were administered BPA (40 µg/kg/day) during pregnancy or lactation. An intruder test revealed in males but not in females an increase in defensive behavior due to BPA. We studied the effect of BPA on sexual behavior by testing sexual orientation and sexual activity. Male sexual orientation toward a stimulus female was not affected by BPA, whereas the sexual activity test revealed a slight impairment of sexual performance due to BPA in terms of latency and frequency of intromissions. In females, BPA produced a small increase in sexual motivation and receptive behavior. In conclusion, BPA administration, both during pregnancy and during lactation, does not masculinize female behavior or potentiate masculinization processes of males. On the contrary, we observed a potentiation of female behavior in females and a depotentiation of male behavior in males. *Key words:* **bisphenol A, environmental estrogens, rat, sexual behavior, sexual orientation, social behavior.** *Environ Health Perspect* **110(suppl 3):409–414 (2002).** ***http://ehpnet1.niehs.nih.gov/docs/2002/suppl-3/409-414farabollini/abstract.html***

In mammals, the action of estrogen or aromatizable androgen on the brain during the perinatal period has a subsequent effect on sexual behavior (*1,2*). This suggests that environmental estrogens could act on behavior through a similar mechanism. The expectation in this case is that early contact with environmental estrogens, in certain temporal windows and at particular doses, could result in dramatic changes in female and male behavior. It has been shown in litter-bearing mammals that even small physiological changes of the hormonal milieu during pregnancy can affect adult sociosexual behavior and morphological reproductive parameters (*3*).

Bisphenol A (BPA) is a compound that is widespread in human environments, used in the food industry, as well as in dentistry (*4,5*). It is known to have a weak estrogenic action (*6–8*). Low doses of BPA administered perinatally can modify nonsocial behavior in rats (*9*) and can advance puberty in female mice (*10*), although some effects are controversial (*11*).

In this study, we investigated the effects on behavior of perinatal administration of 40 µg/kg BPA, a low, environmentally compatible dose. This dosage proved, in a previous study with two dosages of BPA (40 and 400 µg/kg), to be effective on nonsocial behavior parameters (*9*). We considered two temporal modalities of administration: prenatal and postnatal. Both of them are potentially critical for sexual differentiation of the brain in the rat (*12,13*). We administered BPA to both pregnant or lactating mothers and studied the effects on sexual and related behaviors in females and males through a battery of tests able to reveal fine alterations of behavior: the intruder test, the sexual orientation test (SOT), and the sexual activity test, applied in sequence.

Materials and Methods

Subjects

We used 72 mature Sprague-Dawley rats (36 females and 36 males) born and bred at the Human Physiology Institute, University of Siena (Siena, Italy), that were the offspring of mothers treated during gestation or lactation, as described below. Subjects were organized into three groups: prenatal (PRE), 12 females and 12 males, exposed to BPA during intrauterine development; postnatal (POST), 12 females and 12 males, exposed to BPA during lactation; and control (OIL), 12 females and 12 males, offspring of vehicle-treated mothers. The animals were housed in groups of four, of the same sex and treatment, in polysulfaone (Tecniplast, Malta, Italy) cages (60 × 37 × 20 cm), under a reversed light–dark cycle (dark 0730–1930). Food and water were available *ad libitum*.

Treatment Procedure

We randomly allocated 20 females of reproductive age to two treatment groups: BPA (n = 7) and OIL (n = 13). The two groups were treated daily from mating until weaning of the pups with, respectively, 40 µg/kg BPA and arachis oil. BPA (Fluka Ltd., Buchs, Switzerland) was dissolved in arachis oil at a concentration of 10 µg/mL and administered orally with a pipette. Because the animals enjoyed receiving the oil, the procedure was not stressful. Females were housed for 48 hr with a sexually mature male and then transferred to single cages. On day 2 after delivery, the litters, culled to 4 females and 4 males, were cross-fostered between mothers of the appropriate treatment. Using this procedure, we obtained three groups of pups: *a*) prenatal, pups born from BPA-treated mothers and reared by OIL-treated adoptive mothers; *b*) postnatal, pups born from OIL-treated mothers and reared by BPA-treated adoptive mothers; *c*) control, pups born from OIL-treated mothers and reared by OIL-treated mothers.

Litters were weaned on day 21 and housed until day 45 in the same cage. On day 45, within each treatment group, animals of the same sex were randomly chosen from different litters and housed four per cage, such that no cage contained siblings.

Behavioral Testing

Behavioral testing started at 100 days of age. We first tested the rats for agonistic behavior (intruder test) and then, 1 week later for sexual orientation and sexual behavior. In males, we immediately followed the SOT with the sexual activity test, whereas we performed the latter after a 1-week interval in females. All tests were performed during the dark phase (0900–1300) under dim red light combined with low indirect white light. All sessions were recorded with a video camera (Sony AVC-D5CE, No 17783; Tokyo, Japan); for each test, the video recordings were later analyzed with Noldus Observer software (version 3.0; Noldus Information Technology, Wageningen, Netherlands) by an observer blind to treatment.

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Intruder test. Each experimental animal was matched against an unfamiliar intruder of the same sex and body weight in its own cage for 15 min. The frequency (*f*), duration (*d*), and latency (*l*) of behaviors were recorded in the following behavioral categories (*14*):

- Offensive behaviors (*f, l*): aggressive grooming, threat, offensive sideways posture, offensive upright, chase, attack, bite, full aggressive posture
- Defensive behaviors (*f, l*): retreat, flight, crouch, defensive sideways posture, defensive upright, full submissive posture
- Ambivalent (*f, d, l*): boxing

In addition, we computed the frequency and duration of total agonistic behavior (defensive + offensive + ambivalent), as well as the percentage of defensive, offensive, and ambivalent behavior with respect to total agonistic behavior.

We took vaginal smears from experimental females at the end of the test and considered only females in diestrus for the statistical analysis.

Sexual orientation test. We carried out the test in a black Perspex box (80 × 80 × 35 cm) with two small wire-mesh cages (16 × 12 × 14 cm) fixed on opposite sides. We housed two stimuli—a sexually experienced male and a receptive female—in the lateral cages. (Receptive females had been ovariectomized; receptivity was induced by combined treatment with 5 µg estradiol benzoate 48 hr before testing and 0.5 mg progesterone 4 hr before testing.) Each experimental rat was placed in the center of the arena. During the 5-min test, we recorded the duration of visits to an area (30 × 15 cm) in front of each stimulus animal and the time spent investigating it.

Male sexual activity test. At the end of the SOT, we tested each experimental male in the following way: we removed the stimulus male, placed a circular arena (50 cm diameter) in the center of the apparatus, and placed the experimental male in it together with the same receptive stimulus female used in the SOT. We recorded the number of mounts, number of intromissions, latency to first intromission, latency to ejaculation (the time from the first mount to ejaculation), and refractory period (time from ejaculation to the next mount). We also recorded the duration of genital sniffing of the partner. We stopped the test at the end of the first refractory period, or after 15 min if no mount was displayed, or after 30 min if the animal did not ejaculate.

Female sexual activity test. One week after the SOT, each female was tested for sexual activity in a black Perspex arena (80 × 80 × 35 cm) with a small cage (25 × 12 × 12 cm) fixed inside. The small cage was made of transparent Perspex and had two holes (diameter, 5 cm), large enough to allow passage of the female but not of the male. Before the test, females were allowed to become familiar with the small cage, including passing through a hole. We then performed the test for 20 min: we put the female in the small cage and placed the stimulus male (a sexually mature, experienced subject) in the center of the arena. We recorded the number of lordosis posture, frequency of proceptive behaviors (hopping, darting, ear wiggling), and the time from the beginning of the test to the exit from the small cage (exit latency). We also recorded mounts by the males and calculated the lordosis quotient [(no. lordosis/no. mounts) × 100, taking into account only mounts > 1]. We took vaginal smears from the experimental females before the test.

Statistical Analysis

We processed the data from the intruder test by one-way analysis of variance (ANOVA) for the treatment factor (three levels) and analyzed sexual orientation by two-way ANOVA for treatment (three levels) and stimulus (two levels, male and female). We analyzed male sexual behavior by one-way ANOVA for treatment (three levels) and female sexual behavior by two-way ANOVA for treatment (three levels) and cycle (two levels—diestrus and proestrus).

We applied post hoc analysis (Fisher least significant difference test) for comparison between groups. For the intruder test, we carried out comparisons between numbers of animals by the Fisher exact test.

Animal Welfare

Experimental procedures followed the regulations of the European Communities Council Directive 86/609/EEC (*15*).

Results

Females

Perinatal treatment with BPA had no effect on any of the female agonistic behaviors during the intruder test (one-way ANOVA); we considered only diestrus females (25 of 36) (Table 1). Two-way ANOVA of the SOT data (Table 2) revealed no significant differences for treatment. Preference for the stimulus male was evident for all parameters considered.

Sexual behavior of diestrus and proestrus females was analyzed by pooling the PRE and POST groups (as they did not differ significantly) to test for possible effects despite the small sample. Table 3, showing results of

Table 1. Intruder test: behaviors shown by female rats (mean ± SE).

	OIL (*n* = 9)	PRE (*n* = 8)	POST (*n* = 8)
Agonistic (*f*)	11.0 ± 5.6	9.3 ± 3.6	12.3 ± 3.9
Agonistic (*d*)	19.3 ± 7.4	21.4 ± 7.4	26.6 ± 8.2
Offensive (*f*)	6.9 ± 5.2	5.0 ± 3.4	7.1 ± 3.0
Offensive (*l*)	459.9 ± 114.4	743.9 ± 102.2	479.2 ± 124.7
Defensive (*f*)	2.1 ± 0.9	1.8 ± 0.7	2.1 ± 1.0
Defensive (*l*)	523.3 ± 123.1	539.5 ± 119.7	581.7 ± 139.4
Ambivalent (*f*)	2.0 ± 0.7	2.5 ± 1.1	3.0 ± 1.3
Ambivalent (*l*)	524.4 ± 126.0	555.2 ± 104.3	356.3 ± 120.3
% Offensive/agonistic	45.5 ± 14.1	22.2 ± 14.7	45.2 ± 12.9
% Defensive/agonistic	31.0 ± 14.0	47.5 ± 16.1	29.5 ± 12.9
% Ambivalent/agonistic	23.5 ± 11.3	30.4 ± 13.2	25.4 ± 11.7

Abbreviations: *d*, duration (sec); *f*, frequency; *l*, latency (sec); *n*, number of rats in each group.

Table 2. Sexual orientation in female rats (mean ± SE).

	Time in front (sec)		Time investigating (sec)	
	Female stimulus	Male stimulus	Female stimulus	Male stimulus
OIL (*n* = 12)	45.4 ± 5.9	107.5 ± 9.2	28.8 ± 4.7	65.4 ± 8.5
PRE (*n* = 12)	47.6 ± 4.3	105.6 ± 5.7	29.6 ± 3.4	59.9 ± 3.9
POST (*n* = 12)	53.3 ± 3.7	121.5 ± 9.0	35.1 ± 2.8	72.7 ± 5.9

n, number of rats in each group.

Table 3. Two-way ANOVA applied to sexual behavior parameters in female rats (*F* and *p* values).

	Treatment		Cycle		Treatment × cycle	
	F	*p*	*F*	*p*	*F*	*p*
df = 1, 32						
Exit (latency)	3.95	0.055	0.60	NS	0.00	NS
Proceptive behavior (*f*)	0.08	NS	11.11	<0.002	0.08	NS
Lordosis (*f*)	4.16	<0.05	12.60	<0.001	4.30	<0.04
df = 1, 14						
Lordosis quotient	0.00	NS	117.7	<0.0001	0.39	NS

Abbreviations: df, degree of freedom; NS, not significant.

the two-way ANOVA, indicates that perinatal BPA administration modified the exit latency and number of lordosis posture. Cycle was significant for number of lordosis posture, lordosis quotient, and frequency of proceptive behavior. The treatment × cycle interaction was significant only for number of lordosis posture. Exit latency was decreased in the BPA group, independently of the cycle phase (Figure 1). In proestrus females, lordosis was increased by BPA (Figure 2), whereas proceptive behavior (Figure 3) and lordosis quotient (Figure 4) were not influenced by the treatment.

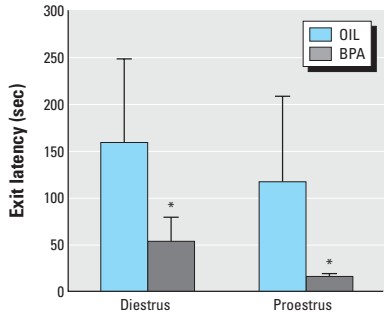

Figure 1. Effects of perinatal exposure to BPA on exit latency in female rats (mean ± SE). *p = 0.05, compared with control group.

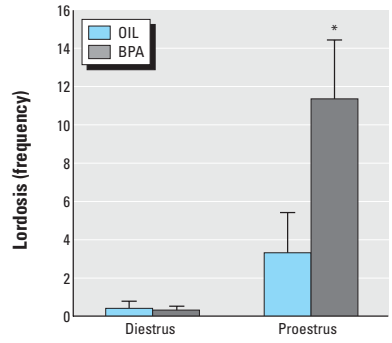

Figure 2. Effects of perinatal exposure to BPA on number of lordosis posture in female rats (mean ± SE). *p < 0.05, compared with control group.

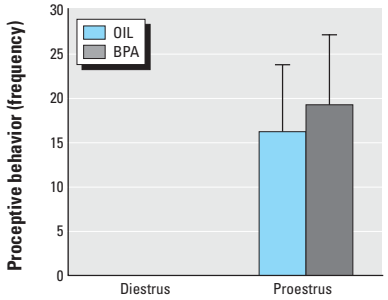

Figure 3. Effects of perinatal exposure to BPA on proceptive behavior in female rats (mean ± SE).

Males

In the intruder test the proportion of animals showing defensive behavior was increased, whereas the number showing ambivalent behavior was decreased by prenatal exposure to BPA (Table 4). Analysis of the behavioral elements, with one-way ANOVA followed by the least significant difference test, showed that the ratio of defensive behavior to total agonistic behavior was significantly increased (F = 4.14, p < 0.02, df = 1, 33) only in the PRE group (Table 5). Preference for the stimulus male or female in the SOT was not affected by BPA; however, this experiment did not reveal a clear sexual preference in controls (Table 6). In the sexual behavior test, the analysis was restricted to males achieving ejaculation (PRE, n = 10 of 12; POST, n = 10 of 12; OIL, n = 12 of 12). One-way ANOVA applied to the sexual activity data revealed differences due to treatment for the number of intromissions necessary to reach ejaculation (F = 3.24, p = 0.054, df = 2, 29) and latency to first intromission (F = 6.44, p < 0.005, df = 2, 29). Genital sniffing of females was also affected by treatment (F = 5.15, p < 0.01, df = 2, 29).

An increase of the number of intromissions (Figure 5) due to perinatal exposure to BPA was evident only in the POST group. We found the other significant effects only in the PRE group: increased latency to intromission (Figure 6) and genital sniff duration (Figure 7). Number of mounts (Figure 8), latency to ejaculation (Figure 9), and refractory period (Figure 10) were not significantly affected.

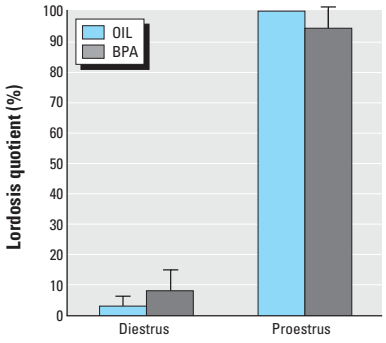

Figure 4. Effects of perinatal exposure to BPA on lordosis quotient in female rats (mean ± SE).

Discussion

Females

The female reaction to intruders of the same sex was not influenced by either pre- or postnatal BPA administration. The intruder test did not reveal an increase of aggressiveness or other changes of social behavior (Table 1). The lack of significance of the effect of treatment could be due to the low level of agonistic interactions observed in the test. Moreover, in this test the stimulus females were intact females not controlled for the cycle phase; this could increase the variability of the behavior of the experimental females.

Table 4. Intruder test: number of male rats displaying aggressive behavior (n = 12).

	OIL	PRE	POST
Agonistic	10	10	10
Offensive	9	5	9
Defensive	4	9*	6
Ambivalent	8	3*	6

n, number of rats in each group. *p < 0.05 compared with control group (Fisher's exact probability test).

Table 5. Intruder test: behaviors shown by male rats (mean ± SE, n = 12).

	OIL	PRE	POST
Agonistic (*f*)	16.6 ± 5.4	9.7 ± 2.9	15.4 ± 5.5
Agonistic (*d*)	47.1 ± 16.0	31.0 ± 10.3	78.4 ± 40.0
Offensive (*f*)	12.5 ± 4.5	3.0 ± 1.4	9.1 ± 4.2
Offensive (*l*)	467.9 ± 81.5	659.0 ± 95.7	553.0 ± 76.4
Defensive (*f*)	2.2 ± 1.0	5.7 ± 1.9	5.1 ± 3.1
Defensive (*l*)	714.9 ± 89.5	407.2 ± 98.5	586.8 ± 100.5
Ambivalent (*f*)	1.9 ± 0.6	1.0 ± 0.6	1.3 ± 0.6
Ambivalent (*l*)	445.4 ± 98.7	715.5 ± 97.3	693.9 ± 76.2
% Offensive/agonistic	51.1 ± 11.6	21.8 ± 10.1	45.5 ± 12.4
% Defensive/agonistic	15.1 ± 7.7	57.4 ± 12.8 *#	26.4 ± 11.1
% Ambivalent/agonistic	17.1 ± 8.1	4.2 ± 2.6	11.4 ± 6.3

n, number of rats in each group. Post hoc comparisons (Fisher least significant difference test): *p < 0.05 compared with control group; #p < 0.05, comparison between BPA groups.

Table 6. Sexual orientation in male rats (mean ± SE, n = 12).

	Time in front (sec)		Time investigating (sec)	
	Female stimulus	Male stimulus	Female stimulus	Male stimulus
OIL	94.6 ± 11.9	88.7 ± 7.4	51.7 ± 5.3	53.6 ± 5.2
PRE	90.3 ± 5.2	93.3 ± 7.0	57.8 ± 4.6	56.1 ± 4.8
POST	83.8 ± 8.1	103.6 ± 9.7	57.9 ± 6.8	58.3 ± 7.1

n, number of rats in each group.

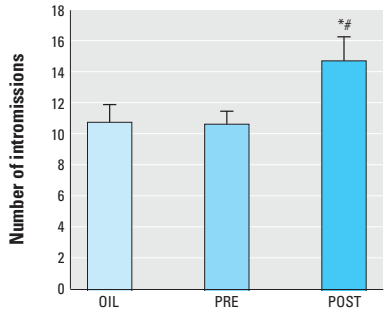

Figure 5. Effects of prenatal or postnatal exposure to BPA on number of intromissions in male rats (mean ± SE). *$p < 0.05$, compared with control group; #$p < 0.05$, comparison between BPA groups.

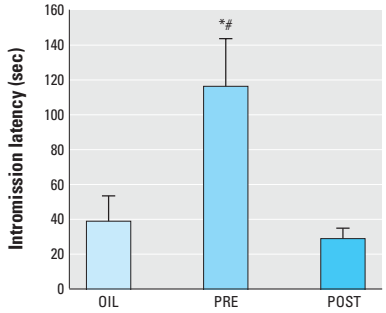

Figure 6. Effects of prenatal or postnatal exposure to BPA on intromission latency in male rats (mean ± SE). *$p < 0.05$, compared with control group; #$p < 0.05$, comparison between BPA groups.

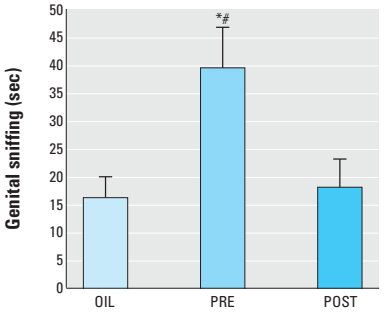

Figure 7. Effects of prenatal or postnatal exposure to BPA on duration of genital sniffing in male rats (mean ± SE). *$p < 0.05$, compared with control group; #$p < 0.05$, comparison between BPA groups.

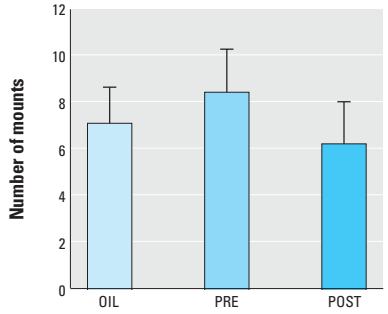

Figure 8. Effects of prenatal or postnatal exposure to BPA on number of mounts in male rats (mean ± SE).

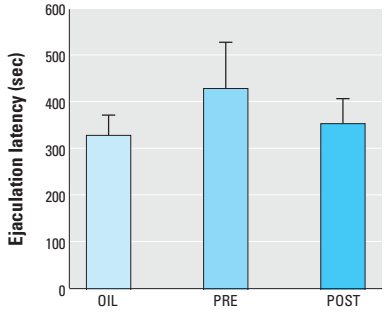

Figure 9. Effects of prenatal or postnatal exposure to BPA on ejaculation latency in male rats (mean ± SE).

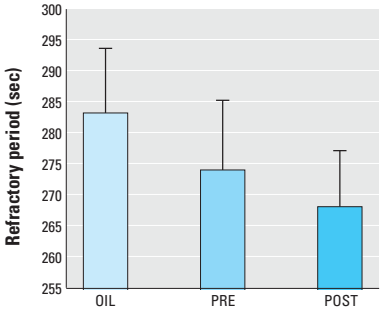

Figure 10. Effects of prenatal or postnatal exposure to BPA on refractory period in male rats (mean ± SE).

This lack of effect of BPA was confirmed by the SOT, in which treated and control females showed the same orientation toward stimulus males and females (Table 2). In the SOT, we used ovariectomized, hormone-primed females as the stimulus.

The sexual behavior test revealed some effects of BPA, only marginally significant, probably because of the small sample: because the effects of pre- and postnatal treatment did not differ significantly for any behavior, we pooled the PRE and POST data. This revealed in proestrus females treated with BPA a significant increase of receptive behavior (lordosis frequency) (Figure 2) and an increase of sexual motivation (exit latency) (Figure 1) in both phases of the cycle. Proceptive behavior was unaffected by BPA (Figure 3).

In females, the direction of the effect produced by early BPA administration is not in line with the expectation of a masculinization/defeminization of the brain due to an estrogenic action of this substance: this expectation was based on the fact that α-fetoprotein does not bind to BPA to the same degree that estradiol does (*16*). This could allow a low concentration of BPA to pass into cells and act at the genomic level. On the contrary, we observed a slight intensification of sexual behavior.

Males

As with the females, the male reaction to intruders of the same sex was not influenced by either pre- or postnatal BPA administration, regarding aggressiveness. In contrast, the percentage of defensive elements with respect to total agonistic elements was increased in the PRE group, as was the proportion of animals showing defensive and ambivalent behaviors. In the SOT we observed no difference in orientation toward the stimulus male or female in control rats; consequently, no difference in sexual orientation was detectable between treated and control animals, thus masking possible effect of BPA on sexual motivation. The sexual behavior test revealed a general impairment of sexual performance due to BPA. This effect is in the direction of a reduced performance, expressed in the PRE group by increased latency of intromission (Figure 6) accompanied by increased duration of genital investigation (i.e., premating activity) (Figure 7), and in the POST group by increased number of intromissions necessary to reach ejaculation (Figure 5). This pattern could indicate an impairment in the timing of copulatory sequence, especially in the PRE group. On the whole, however, male sexual behavior was not disrupted by the treatment: such important measures of male sexual activity as latency of ejaculation (Figure 9) and refractory period (Figure 10) were not significantly affected.

In males the direction of the observed effect of early BPA administration is not congruent with the concept that estrogen or aromatizable androgens can masculinize the brain; in contrast we observed a slight impairment of sexual performance. However, a masculinizing effect is far more difficult to detect in males than in females. In intact males, results from research on the early effects of gonadal steroids are not easy to understand (*3*). For instance, in intact male rats treated postnatally (2 and 5 days of age) with estrogen, Feder (*17*) observed an impairment of sexual performance that parallels our observations.

Conclusions

We were not able to identify a systematic difference between pre- and postnatal treatment. BPA may act on different subsystems underlying behavior, each with distinct temporal characteristics of sensitivity. In males the slight changes observed point to an effect of prenatal BPA in inducing more

defensive-type strategies during agonistic encounters and in slowing the copulatory sequence, but we also observed a reduction of sexual performance in postnatally treated animals.

On the whole, in both sexes, sexual activity seems quite sensitive to perinatal exposure to BPA, in line with recent research showing significant effects on behavior and on reproductive indices after early BPA administration (*9,10,18,19*). The direction of the observed modifications in the two sexes in our experiment suggests that the effect of early BPA exposure on adult behavior may not be mediated by a classic organizational process activated by estrogenic action. This could be explained by a different affinity of BPA (with respect to estradiol) with estrogen α-receptors (*8*) and β-receptors (*20,21*). Our previous experiments have demonstrated that exposure of female rats in adulthood to BPA at the same dosage as in the present experiment, modifies the number of estrogen α-receptors in the brain, thus proving an effect on the central nervous system (*22*).

As for the weak effects observed, in socioecological terms even slight changes in the sphere of sexual behavior may have important consequences in terms of fitness and welfare at the individual level with consequences on population dynamics.

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